Characterization of an Italian landrace of Cyclanthera pedata (L.) Schrad. of herbal and horticultural interest

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Abstract

Caigua (*Cyclanthera pedata* (L.) Schrad.) is a native species of the Andes whose fruits are recently gaining attention as herbal and healthy product. A genotype of Caigua traditionally cultivated in Camonica Valley (Italian Alps), called “Ciuenlai” (or “Milione/Milioncino”), is almost unknown. For this reason, the phytochemical, nutritional and ecological (functional strategy) characterization of “Ciuenlai” was carried out. Phytochemical and nutritional analyses were performed considering its fruits, leaves, sprouts, and seeds, and compared with commercial South American plant material. Fruits of “Ciuenlai” showed a similar content of phenolic compounds but a lower content of saponins and flavanones and dihydroflavonols than the comparison samples. Conversely, the content in caffeoylquinic acid (frequently considered a quality marker for herbal use) was four times higher for “Ciuenlai” (0.64 ± 0.04%) comparing with the commercial genotype grown in the same area (0.15 ± 0.01%). Interestingly, leaves and sprouts of “Ciuenlai” resulted rich in secondary metabolites, including saponins and flavanones and dihydroflavonols, suggesting that these parts could be eligible for herbal use. Considering the ecological and agronomical features, “Ciuenlai” showed a more ruderal strategy, and different growth, producing more fruit earlier in the season, much smaller than the South American genotype. “Ciuenlai” could have developed an ecological adaptation to the peculiar climatic conditions of the mountain areas of Camonica Valley, different from the native area. The herbal use of “Ciuenlai” could be a useful strategy for its valorisation, other than the promotion of this product as typical food.

Introduction

Caigua (*Cyclanthera pedata* (L.) Schrad.) is an herbaceous plant of Cucurbitaceae family native of the Andean territories of South America (Klien et al. 1989). This plant is called with different other names, as achoccha, quishiu, caiba, caifa or kaiwa in Mexico and Bolivia, pepino-do-ar, pepino-de-porco, pepino-de-comer, chuchu-paulista e taiuiá-de-comer or maxixe do reino in Brasil and Achocha (quechua) or Achokcho in Argentina (Klien et al. 1989). This non-conventional vegetable was cultivated by the Incas who utilized its fruits as food (Dietschy 1953; Popenoe 1990; Fernández Honores and Rodríguez Rodríguez 2007). It is an annual climbing plant provided with tendrils, palmate leaves, and small unisexual flowers (monoecious species) at the leaf axilla. The fruit is a green-pale/green ovoid pepo with grooves (Macchia et al. 2009), smooth or with soft spines. The mesocarp is thin and succulent while the endocarp is white and fluffy when mature, the inside of the ripe fruit is hollow, and contains dark-brown seeds roughly quadrangular attached to a placenta. It can be found in the Andes range under spontaneous or sub-spontaneous condition (Fernandes et al. 2005), from Bolivia to Panamá and in the mountain region of Central America and México (Pittier 1910). It bears well extreme temperatures (hot and cold), from sea level to more than 2,500 m a.s.l, and currently Caigua is cultivated in various parts of the world where it was introduced as Africa, China, Nepal, and Europe. In Italy this species has been traditionally cultivated, for at least 50 years, only in few Alpine Valleys of Lombardy region (Northern Italy), as Camonica Valley, where the vegetable takes the name of “Ciuenlai” (Bissanti 2020; Rossi et al. 2019; Giupponi et al. 2020), or “Milione”, “Milioncino” (Fig. 1). “Ciuenlai” is cultivated by very few farmers (mostly hobbyists) and was not affected by improvement programs. Therefore, it is considered a landrace of Lombardy (Rossi et al. 2019; Giupponi et al. 2020).

The Caigua fruit is edible both raw and cooked: the mature fruits are cooked and can be filled with meat or fish, while young and immature fruits are consumed raw for example in salads. The sprouts and the young leaves are edible as well. Among the recipes based on Caigua there are different foods of Creole origins such as Guiso de Caigua or Caigua Rellena. The most used part of the plant, however, are fruits (Monigatti et al. 2013), that are also the most studied part of the plant, due to their content of secondary metabolites including phenolic compounds, flavonoids, coumarins, tannins, terpenes, and other minority compounds (Macchia et al. 2009). Several compounds belonging to the class of saponins and flavonoids glycosides were isolated from the fruits (De Tommasi et al. 1999; Montoro et al. 2001), while many cucurbitacins were identified and isolated from the seeds (De Tommasi et al. 1996).

Some traditional uses as herbal remedy are also known. The fruit juice or infusion is recommended as a treatment for people who have high blood cholesterol levels, hypertension, arteriosclerosis, circulatory problems, and diabetes. Fruit and leaves boiled in olive oil are used externally as a topical anti-inflammatory and analgesic. Dried and powdered seeds shall be taken as remedy for intestinal parasites. Leaves are considered hypoglycaemic and are prepared in a decoction for the treatment of diabetes (Antih et al. 2016). Recently, Caigua has gained attention in the pharmaceutical field (Frigerio et al. 2021). Different effects are attributed to this plant, the most interesting being the hypertension inhibition (Ranilla et al. 2010) hypoglycaemic and hypocholesterolemic properties (Egg 1999; Gonzales et al. 1995; Ranilla et al. 2010), other than analgesic and antioxidant (Vásquez 2003; Garcìa et al. 2010). Caigua is of interest for herbal use as a dried drug in the preparation of forms of solid dosage (tablets or capsules), for its transformation into extracts and in the preparation of food supplements or other health products or nutraceuticals.

However, to date very little is known about the “Ciuenlai” landraces and European herbal industry uses only imported product from America. The part used in the extract is only the fruit, while sprouts and leaves have currently no use, although they represent most of the aboveground plant biomass. At the present, no official methods are used for the quality control of the plant and its products; and the authentication of commercial samples of *C. pedata* is generally carried out using generic procedures performed by thin layer chromatography (Carbone et al. 2004). The percentage content of total caffeoylquinic acids expressed as chlorogenic acid is used as parameter from the herbal industry to evaluate the quality of dried fruits. The “Ciuenlai” could be an alternative as raw material, but phytochemical studies are needed to evaluate the possibility of using it. In addition, the evaluation of its ecological characteristics (such as the functional strategy) and some agronomic characteristics would provide a better knowledge of the features of this landrace and facilitate its conservation and/or cultivation.

The aim of this study is to investigate if the “Ciuenlai” has distinctive phytochemical and nutritional features comparing with the commercial South American genotype and the imported product. The phytochemical and the nutritional profiles of the fruits were determined to make a preliminary evaluation of the potential of the "Ciuenlai" for the production of raw material for herbal use in mountain areas of the Italian Alps. The aerial parts of the plant (leaves and sprouts) and the seeds were also included in the phytochemical analysis to evaluate their potential as raw material for the same usage. Additionally, functional strategy, according to the theory of Grime (1974, 1977, 2001), and agronomical evaluation of the "Ciuenlai" were performed and a brief historical framework and the possible strategy of valorisation of this product were discussed.

Materials And Methods

Plant material

Three genotypes of Caigua were included in the experimental design. The "Ciuenlai" (A) was cultivated starting from seeds from a local farmer (Oriana Belotti) growing it from approximately 50 years in the Commonality of Esine (Camonica Valley; Latitude 45°55'35,76" N; Longitude 10°15'6,84" E), who reported to have received the seed from an older fellow villager. Commercial South America genotype (B) was cultivated from seeds purchased from Rarepalmseeds seller (Muenchen, Germany). The genetic identity of both the commercial and "Ciuenlai" seeds was checked by means of DNA barcoding techniques by the centre FEM2-Ambiente of University of Milano-Bicocca, and the "Ciuenlai" was confirmed belonging to the species *Cyclanthera pedata* (Frigerio et al. 2021). A sample of dried fruit and seeds of imported Peruvian Caigua (C) was purchased from Herbaperu importer (Wrocław, Poland) and was included in the analysis as comparison.

Experimental fields were settled in Camonica Valley, in the municipality of Esine (latitude 45°55'35,76" N, Longitude 10°15'6,84" E, elevation: 286 m a.s.l.) in 2021. This area belongs to the Temperate Oceanic bioclimate (Rivas-Martinez and Rivas-Saenz, 2009). It has a rainfall of 1.100 mm per year, concentrated mainly in the spring and the autumn; the annual average temperature is about 8,9°C; minimum temperatures and precipitation are during the winter months (data source: Centro Meteo Lombardo). According to Blasi et al. (2014), the area is within the North-eastern Alps Ecoregional Subsection (Central and Eastern Alps Section, Alpine Province, Temperate Division).

Soil was prepared at the end of March ploughing at depth of about 20–30 cm, manual removal of weed roots, and successive mechanical milling. Identical fertilization conditions throughout the field experiments were employed, with the addition of about 15 kg of green mulch prior to cultivation. Nets/trellises on which the plant can climb were set up. Three-four seed were planted directly in the soil at the depth of 2,5 – 4 cm at the end of April, selecting the most vigorous at sprouting, and then leaving the plants on a single row, with one meter between plants and two meters between rows. A total of 200 plants were grown for each genotype. Plants were irrigated constantly, each time leaves looked withered.

For genotypes A and B, the mean germination time in days was fixed when approximately the 60% of planting locations presented at least one seed germinated, while the mean flowering time was fixed when at least 60% of plants produced the first flower.

Fruits were manually harvested and measured (weight and size). Production was registered from end of August to the end of September, even if plants went on producing until early November, also if with a decreasing trend and facing several frost events. The average production of fruit was calculated considering only the productive plants and by calculating the mean of production/plant (mean number of fruits per plant). Fruits, leaves and shoots were collected along several points of the fields, then they were mixed to randomize. The plant material collected was then dried in ventilated ovens at 70°C (MPM Instruments M120-VF) for 36 hours for sprouts and leaves and 48 hours for fruits. Ripen fruits were split in quarts, the seeds removed and dried separately while immature fruits were split in quarts and dried without removing the seeds and the pulp. The degree of ripeness was discriminated following the criteria of the complete detachment of the seeds from the pulp. If this happened, the fruit was categorized as ripened, while if seeds were still of a light colour and not detached from the pulp, fruit was considered immature.

Dried plant material was stored in sealed glass jar and kept in the dark at room temperature prior the analysis. The samples for the analysis were pulverized using MM400 vibrational mill (frequency: 30 Hz; time: 1 minute), then used immediately for the assays.

Phytochemical and nutritional analysis

Chemicals and reagents

All the solvent used throughout the experiments were of analytical grade purity, while water was of HPLC grade purity. All the solvents and reagents were purchased from Merck (Milan, Italy) and used without further purification.

Extraction and analysis through UV-Vis Spectroscopy

The ethanol extracts of the pulverized plant material were prepared adapting the procedure reported by Tan et al. (2014). Briefly, an exactly weighed sample (1 g) was transferred in conical-bottom centrifuge tube, suspended in 20 mL of 70% ethanol (v/v) and stirred at room temperature for 48 hours. After that, the supernatant was collected by centrifugation at 4000 rpm for 5 min. The pellet was washed thrice with 5 mL of 70% ethanol and centrifuged. The collect supernatants were evaporated to dryness by rotary evaporation at 50°C and the residue was dissolved with 70% ethanol adjusting the final volume to 5 mL. The obtained ethanol extract was centrifuged at 4000 rpm for 15 min and stored at -20°C prior the analysis. All the spectroscopic analysis were performed on Varian Cary 50 scan Agilent 5301 (Santa Clara, CA, USA). All the assays were performed in triplicate.

The total phenols content was determined as described by Rivas et al. (2013) using the Folin-Ciocalteau's method with minor modifications. Briefly, 0.1 mL of ethanol extract were diluted with 6 mL of water in a conical bottom centrifuge tube. Samples of leaves and sprouts extracts were diluted 1/10 with 70% ethanol prior the analysis. Then, 0.5 mL of Folin-Ciocalteau's reagent 2 N were added, and the resulting mixture was mixed and let stand for 2 min. After that, 1.5 mL of 20% sodium carbonate solution (w/v) were added followed by 2 mL of water. The resulting solution was mixed and incubated for 2 hours in the dark at room temperature. The absorbance was measured at 760 nm against a blank prepared using 0.1 mL of 70% ethanol. Gallic acid solutions with concentrations ranging from 125 to 900 mg/L were used to build a calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g).

The total flavones and flavonols content was determined as described by Rivas et al. (2013) using the aluminium chloride method with minor modifications. Briefly, 0.5 mL of ethanol extract was mixed with an equal volume of aluminium chloride hexahydrate 2% (w/v) in ethanol. Samples of leaves and sprouts extracts were diluted 1/1000 with 70% ethanol prior the analysis. The resulting solution was incubated for 60 min in the dark at room temperature, and the absorbance was measured at 420 nm against a blank prepared using 0.5 mL of 70% ethanol. Quercetin solutions with concentrations ranging from 5 to 25 mg/L were used to build a calibration curve. The total flavones and flavonols content was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g).

The flavanone and dihydroflavonols content was determined as reported by Rivas et al. (2013) using the 2,4-DNPH method with minor modifications. Briefly, an aliquot of the ethanol extract with volume ranging from 0.02 mL to 0.05 mL was diluted with absolute ethanol to a final volume of 0.25 mL. Then, 0.5 mL of 2,4-dinitrophenylhydrazine (2,4-DNPH) solution (prepared dissolving 1 g of 2,4-DNPH in 2 mL of concentrated sulphuric acid and diluting with methanol to a final volume of 100 mL) were added and the resulting mixture was incubated at 50°C for 50 min. After that, the mixture was let to cool down to room temperature and 0.3 mL of the resulting solution were mixed with 0.7 mL of 10% potassium hydroxide methanol solution (w/v). The resulting dark mixture was centrifuged at 1500 rpm for 10 min, and 0.25 mL of the supernatant were diluted with methanol to a final volume of 1.5 mL. Absorbance was measured at 492 nm against a blank prepared using 70% ethanol. Naringenin solutions with concentrations ranging from 250 to 2000 mg/L were used to build a calibration curve. The total flavanones and dihydroflavonols content was expressed as milligrams of naringenin equivalent per gram of dry weight (mg NGE/g).

The total saponins content was determined as reported by Tan et al. (2014) using the vanillin-sulphuric acid method with minor modification. Briefly, 0.1 mL of the ethanol extract were diluted with 70% ethanol to a volume of 1 mL. After that, 0.3 mL of the resulting solution were mixed with 0.3 mL of 8% vanillin ethanol solution (w/v) and 3 mL of 72% sulphuric acid (v/v). The resulting solution was mixed and incubated at 60°C for 15 min and then cooled in iced-cold water. Absorbance was measured at 560 nm against a blank prepared using 70% ethanol. Ginsenoside Rg_1 solutions with concentration ranging from 30 to 450 mg/L were used to build a calibration curve. The total saponins content was expressed as milligrams of ginsenoside Rg_1 equivalents per gram of dry weight (mg GSE/g). Ginsenoside Rg_1 (CAS Number: 22427-39-0) was used as standard because of the high structural similarity with the saponins isolated from the fruit of *C. pedata* by De Tommasi et al. (1999).

The total sugars content was determined accordingly to the procedure reported by DuBois et al. (1956). Briefly, an aliquot of the ripened or immature fruit and seeds extracts were diluted with water to obtain 2 mL total of solution with a dilution in a 1/200 and 1/400 ratio. To the resulting solution, 0.050 mL of a solution obtained by mixing 80 g of phenol with 20 g of water were added. After that, 5 mL of concentrated sulphuric acid were added rapidly to ensure a good mixing of the solution. The solution was allowed to stand for 10 min at room temperature, then vortexed and incubated for 15 min in a water bath at 30°C. The absorbance was measured at 490 nm against a blank prepared using 70% ethanol. Glucose was used as standard, and results were expressed as mg of glucose equivalents per gram of dry weight (mg GE/g).

Assay of content of caffeoylquinic acid

The total content of caffeoylquinic acids was determined adapting the procedure described in the monograph of artichoke dry extract in Official Pharmacopoeia of the Italian Republic XII Edition (Commissione permanente per la revisione e la pubblicazione della farmacopea ufficiale 2008). Briefly, an exactly weighed sample (5 g) of pulverized plant material was extracted with 40 mL of 75% ethanol heating on a water bath at 70°C for 15 min. After that, the suspension was cooled down to room temperature and the supernatant was collected after decantation, and the solid residue was extracted exhaustively repeating the operations thrice with 15 mL of 75% ethanol. The collected ethanol extracts were evaporated to dryness by rotary evaporation at 50°C and the residue was dissolved with water adjusting the final volume to 40 mL. The resulting solution was heated to boiling and 2 mL of aqueous lead acetate saturated solution (prepared by dissolving 51.7 g of lead(II) acetate trihydrate in 100 mL of carbon dioxide-free water) were added. The precipitate was collected by centrifugation and washed with 5 mL of water discharging the supernatant. Then, the precipitate was dissolved with 70 mL of diluted acetic acid (prepared by diluting 12 g of glacial acetic acid to a final volume of 100 mL with water), and the resulting solution was heated to boiling and filtered when hot. To the filtrate, 2 mL of a sulphuric acid solution (200 mL/L) and the resulting mixture was cooled down to room temperature and centrifuged. The clear supernatant was transferred in a 100 mL volumetric flask, and the pellet was re-suspended with 5 mL of diluted acetic acid and centrifuged. The clear solution was transferred in the volumetric flask and made up to volume with diluted acetic acid. 2 mL of the resulting solution were transferred in a 50 mL volumetric flask and made up to volume with methanol. The absorbance of the resulting solution was read at 325 nm against a blank prepared mixing 2 mL of diluted acetic acid with methanol to a final volume of 50 mL.

The percentage content of caffeoylquinic acid (CCQA) expressed as percentage of chlorogenic acid on the dry weight was calculated as follows:

$$CCQA = \frac{\mathrm{A} \times 2500}{485 \times dw}$$

where A is the absorbance of the methanol solution, 485 is the extinction coefficient of chlorogenic acid, and dw is the dry weight of the pulverized plant material in grams.

Other nutritional assays

The total lipids content was determined adapting the improved Bligh and Dyer's method developed by Mubarak et al. (2016). Briefly, an exactly weighed sample (0.5 g) of pulverized plant material was suspended with 1 mL of methanol and 0.5 mL of chloroform and sonicated for 5 min in an iced water bath using Digital Ultrasonic Cleaner MH020S (USA). The suspension was kept 18 hours at room temperature, vortexed for 2 min, 0.5 mL of chloroform were added, and the suspension was vigorously mixed for 1 min. Then, 0.5 mL of water were added, and the suspension were vortexed again for 2 min. The resulting mixture was centrifuged at 4000 rpm for 10 min and the lower organic layer was transferred in a pre-weighed glass vial. 1 mL of chloroform is added, and the procedure was repeated. The two organic layers collected were allowed to evaporate in oven at 80°C to constant weight. The total lipids content was expressed as percentage of dry weight (% Lipids).

The total nitrogen content (% N) was determined by Kjeldahl method accordingly to the AOAC Method 945.18-B. The crude protein content (% Proteins) was calculated as % N × 6.25 (AOAC 2005).

The total ash content (% Ash) was determined accordingly to the AACC Method No.08-01.01. Results were expressed as a percentage of the dry weight (American Association of Cereal Chemists, 1999)

CSR functional strategy evaluation

The analysis of the competitor, stress-tolerator, ruderal (CSR) functional strategy of Grime (1974, 1977, 2001) of the two genotypes of *C. pedata* cultivated in the experimental field (A, B) was performed according to the method proposed by Pierce et al. (2017). In detail, 10 fully expanded leaves of each genotype were collected in the experimental fields during the month of July 2021. The leaf samples were collected from different plants considering those without disease. All the 20 leaf samples collected were wrapped in moist paper and stored in a refrigerator at 4°C for one night. Leaf fresh weight was measured from these saturated organs using analytical weight scale (Precisa XB 220A, 0.0001 g). The leaves were digitized with a digital scanner (Samsung X3280NR) and ImageJ software (Schneider et al. 2012) was used to calculate their leaf area. Leaf dry weight was measured after oven drying (105°C for 24 h). CSR values and functional strategy were determined using 'StrateFy' spreadsheet (Pierce et al. 2017) and were plotted in the CSR ternary graph using the 'ggplot2' package of R software (R Development Core Team 2021).

Statistical analysis

Data were analysed using one-way ANOVA (once the assumptions of normality of group data and homogeneity of variances were verified using the Shapiro-Wilk test and Levene's test, respectively) with Tukey test applied post-hoc. The data were expressed as mean ± standard deviation (SD) and differences were considered statistically significant when $p < 0.05$.

Phytochemical and nutritional data of ripen and unripen fruits were ranked using Principal Component Analysis (PCA) in order to highlight the main variables that differentiated the samples. PCA was performed using Statgraphics 5.1 (STCC Inc.; Rockville, MD, USA).

The data returned by the CSR analysis (CSR ternary coordinates) were analysed using permutational multivariate analysis of variance (PERMANOVA). PERMANOVA was performed using R and considering C, S and R as dependent variables and the genotypes as independent variables.

Historical data collection

Information regarding the introduction, cultivation techniques and uses of "Ciuenlai" was collected by bibliographic research and interviewing elderly farmers from Camonica Valley, especially Mrs Oriana Belotti who is a known custodian farmer. The interview was organized in questions, but the interviewed farmer was let free to divert from the topic if necessary. The main questions were then:

- Age and other personal information
- The common names of the landrace (also in local dialect)
- For how long has been cultivated and where the original seeds/propagation material was obtained
- The peculiarities (shape, taste etc.)
- All the other known custodian farmers and localities of cultivation
- Uses and traditional dishes
- If the plant can be found wild in nature
- Traditional cultivation practices

Results And Discussion

Phytochemical and nutritional analysis of fruits and seeds

Figure 2 shows the phytochemical and nutritional parameters of the samples analysed. Mature fruit of the "Ciuenlai" (A1) showed a content of total phenols (29.66 ± 0.63 mg GAE/g) comparable to the commercial South American genotype grown in the same area (B1: 30.91 ± 2.33 mg GAE/g), while the imported product (C1) showed the highest value (42.02 ± 1.54 mg GAE/g) (Fig. 2a). Phenolic compounds were found to be the main secondary metabolites, in accordance with Rivas et al. (2013). Among phenolic compounds, flavonoids are the main components in Caigua fruits (Carbone et al. 2004; Rivas et al. 2013) Various flavone glycosides were identified for the first time and isolated from Caigua fruit (Montoro et al. 2001) and were consequently proposed as markers for the standardization of Caigua fruits and its transformed products (Carbone et al. 2004). Total flavones and flavonols (Fig. 2b) of Caigua fruits resulted between 12.05 and 7.96 mg QE/g, with the mature fruit of the "Ciuenlai" (A1) showing a content of flavones and flavonols higher than the commercial genotype (B1) and lower than the imported fruit (C1). Mature fruit A1 showed lower content in flavanones and dihydroflavonols (6.54 ± 0.21 mg NGE/g) comparing to B1 (22.61 ± 0.34 mg NGE/g) and C1 (21.76 ± 0.21 mg NGE/g) (Fig. 2c). Values found in this research were higher than the total content of flavanone and dihydroflavonols measured by

Rivas et al. (2013) in extracts of the fresh fruit of Caigua, but this could be due to the different efficiency in the extraction processes.

Mature fruit of the “Ciuenlai” (A1) showed the lowest content in saponins (Fig. 2d). Saponins are amphiphilic compounds containing one or more sugar chains linked to a triterpene or steroidal aglycon. Different pharmacological activities included antidiabetic (Elekofehinti 2015) and cholesterol-lowering (Zhao 2016) properties are attributed to saponins. As mentioned, several compounds belonging to the class of saponins and cucurbitacins were identified and isolated from the fruit and seeds of Caigua (De Tommasi et al. 1999; De Tommasi et al. 1996). The presence of these compounds could therefore explain the cholesterol-lowering and hypoglycaemic properties that are attributed to this plant (Rivas et al. 2013). Caigua is recently finding important applications in the herbal field, in preparations to lower blood levels of cholesterol and fat (Frigerio et al. 2021). Consequently, the dosage of saponins in the fruit of Caigua could be an important parameter to evaluate to determine the quality of fruits for herbal use. However, saponins are also known to be anti-nutrient factors, capable of interfering with some digestive processes and the absorption of various nutrients (Samtiya et al. 2020). In addition, many compounds belonging to this class are characterized by a bitter taste (Price et al. 1985). The lower content in saponins in the “Ciuenlai” could be the result of selection by growers to obtain less bitter fruits. “Ciuenlai”, in Italy, is indeed considered a garden vegetable and not an herbal remedy, differently from what happens in the territory of origin (Antih et al. 2016; Egg 1999; Garcìa et al. 2010). Also, in the indications of local farmers, the fruit is eaten once fully ripened and stuffed, while no other parts of the plant are consumed as food.

The content of total caffeoylquinic acids for fruits was found to be between 0.15 and 0.64% in Caigua fruits samples (Fig. 2e). The “Ciuenlai” resulted the richest in caffeoylquinic acids, four times higher than in the commercial genotype grown in the same area B1 (0.64 ± 0.04%). The content of caffeoylquinic acids is used by some Italian herbal companies as a marker to evaluate the content of active ingredients in the quality assessment of lots of dried Caigua fruits. To be suitable as raw material for extraction and/or other processing, lots must show a content of caffeoylquinic acids not inferior to 0.10% (personal communication of an Italian herbal industry). It is to consider that, despite the remarkable differences, all the different fruits of Caigua analysed could go through the herbal industry.

The mature fruit of A1 had the lowest sugars content (11.93 ± 0.55 mg GE/g) (Fig. 2f). All the samples of ripen fruit considered had less than 5% of lipids, proving Caigua a low-fat food (Fig. 2g). The fruits were also poor in proteins with total nitrogen lower than 1% (Fig. 2h). The total ashes content of ripe fruit was found to be included between 0.65 and 1.20%. The ripe fruit of the “Ciuenlai” showed the greatest content of minerals (Fig. 2i). For most of the nutritional parameters, the unripe fruits of the South American genotype showed higher values than the “Ciuenlai”. The only exceptions were observed for lipids content and total nitrogen.

Seed analysis showed a high content in phenols and saponins(Fig. 2a, 2d), although lower than fruits. The only notable exception is represented by the seeds of the “Ciuenlai” (A3) that have a higher total saponins content than the ripe fruit. From a nutritional point of view, the seeds are rich in lipids with contents ranging from 15.25 to 23.49% on dry weight (Fig. 2g). In particular, the seeds of the "Ciuenlai" (A3) were found to be significantly richer in lipids than the other two genotypes.

In the PCA biplot (Fig. 3) the main variables which diversify the fruit of the three genotypes of Caigua can be observed. In particular, the first axis (PC1) distinguishes the ripened fruit of the "Ciuenlai" (A1) from those the other genotypes (B1 and C1) according to the content of caffeoylquinic acid and the total ashes content. The second axis (PC2) is the one that most diversify the unripen fruit of the "Ciuenlai" (A2) according to the total lipid content and the total nitrogen content.

Phytochemical analysis of leaves and shoots

In Table 1 the phytochemical parameters of the leaves and sprouts of the "Ciuenlai" (A) and of the South American commercial genotype (B) grown in the same area are reported. To the best of our knowledge, this is the first research considering a comprehensive phytochemical composition of the aerial parts of Caigua besides fruits.

Phenolic compounds represent the main secondary metabolites of both leaves and sprouts. Flavones and flavonols content in the leaves of the "Ciuenlai" (A) was lower than in the commercial genotype (B) while flavanones and dihydroflavonols and saponins content was higher in the "Ciuenlai" leaves. No significant difference as found for sprouts between the two genotypes. It is interesting to note that the measured values of total flavones and flavonols for leaves and shoots were found similar to those of the ripe fruits in both genotypes. Our analysis allows only a quantitative comparison and a further research field could be to investigate the composition of the flavonoid fraction of the aerial parts of the plant. Montoro et al. (2005) made a comparison of the flavonoids content of leaves and fruits of Caigua by HPLC analysis coupled to mass spectrometry, finding that the two plant organs have a different qualitative profile.

Regarding the content of caffeoylquinic acids, the leaves of the "Ciuenlai" (A) showed a significantly higher content than those of the commercial genotype (B). Interestingly, leaves of both varieties had a higher content in caffeoylquinic acids than the fruits and shoots had a content even higher than the leaves. The biological significance of caffeoylquinic acids in plant physiology has not been yet fully clarified. Evidence suggests that these compounds have several biological functions, including defence from pathogens and environmental stress. It is also known that these compounds are the key precursors of the biosynthesis of lignin, the main plant support structure (Clifford et al. 2017; Mondolot et al. 2006). The accumulation of caffeoylquinic acids observed for the aerial parts of both genotypes compared to the fruits could be explained by their involvement in lignification processes or defence mechanisms.

Caigua leaves are included in the list of Annex 1 of the Ministerial Decree of 10 August 2018, indicating the plants allowed for herbal use and the preparation of extracts or other health products. Therefore, given the high content of secondary metabolites, the leaves could be interesting as a source of active ingredients or as herbal raw material, also due to the large aerial biomass that the plant produces and that is usually considered a waste product.

Table 1
Results of phytochemical assays on leaves and shoots. Key: A, “Ciuenlai”; B, American genotype. Data with different superscript letter are significantly different ($p < 0.05$)

Plant organ	Sample code	Total phenols content (mg GAE/g)	Total flavone and flavonols content (mg QE/g)	Total flavanones and dihydroflavonols content (mg NGE/g)	Total saponins content (mgGSE/g)	Total caffeoylquinic acids content (%)
Leaves	A	19.88 ± 0.86^{a}	7.76 ± 0.46^{a}	14.02 ± 0.23^{b}	18.55 ± 0.69^{b}	4.24 ± 0.28^{b}
	B	21.01 ± 0.45^{a}	9.18 ± 0.31^{b}	12.34 ± 0.20^{a}	13.34 ± 1.26^{a}	3.16 ± 0.2^{a}
Sprouts	A	22.60 ± 0.26^{a}	7.72 ± 0.05^{a}	8.35 ± 0.31^{a}	12.21 ± 0.72^{a}	6.53 ± 0.02^{a}
	B	22.06 ± 0.23^{a}	7.94 ± 0.23^{a}	8.42 ± 0.71^{a}	12.56 ± 0.29^{a}	6.81 ± 0.16^{b}

Agronomical evaluation and ecological strategy

Both the “Ciuenlai” (A) and commercial South American (B) seeds germinated approximately after 14 days. The South American genotype (B) took about 60 days before producing the first flower, while the “Ciuenlai” (A) one took approximately 21 days. The Commercial genotype produced much more foliage with bigger leaves (Fig. 4), growing higher comparing with the “Ciuenlai”. The “Ciuenlai” fruits were approximately 8 ± 1.4 cm long and with a diameter of 3,5 ± 0.3 cm at the stalk. Commercial fruits were instead bigger, approximately 24 ± 3.5 cm long and with a diameter of 7 ± 0.7 cm at the stalk (Fig. 4). Just about half of the plants of the Commercial genotype were productive (produced at least one fruit), while the “Ciuenlai” plants were all productive. Considering only the productive plants, the South American genotype produced an average of 2.35 fruits per plant, while the “Ciuenlai” produced 21.18 fruits per plant. It is to consider, nevertheless, that the average weight of the fruit for the South American genotype was 97 g/fruit, while for the “Ciuenlai” was 9.8 g/fruit. Because there are evidences of the presence of Caigua in Italy since fifty years at least, we can assume that the species has probably given rise to a smaller variety than the original. The “Ciuenlai” appears more suitable for cultivation in the Alpine and Pre-alpine valleys than the South American genotype, and probably these habitats represent a centre of secondary diversity (Hummer and Hancock, 2015) emerged outside the area of origin of the crop, namely South America. The custodian farmer (Oriana Belotti) refers that Peruvian workers living in Camonica Valley recognized the fruit, although they told to grow a bigger variety in their country of origin. As well the seeds brought in Camonica Valley by them had the same results of the present research, since the plant produced a lot of aerial biomass with very few fruits.

Figure 5 reports the triangular graph provided by CSR functional analysis. The mean CSR strategy of each genotypes (A and B) is C/CR (C:S:R of A = 65:0:35%; C:S:R of B = 85:0:25%) although there are significant differences between the values C and R according to the PERMANOVA test ($F_{1,18}$ = 86.531; $p < 0.01$). In particular, the “Ciuenlai” is more ruderal and less competitive than the South American genotype. In fact, all the “Ciuenlai” samples have C < 70% and R > 30%, while the South American samples have C > 70% and R < 30%. Both genotypes are not stress-tolerators since the S value of all samples analysed is 0%. The analysis of the CSR strategy shown that caigua is a C/CR species that does not tolerate stress intended as all the phenomena which restrict photosynthetic production such as shortages of light, water, and mineral nutrients, or sub-optimal temperatures (Grime 2001). The “Ciuenlai” resulted less competitive than the South American genotype (Fig. 5). While in its area of origin Caigua is found in roadsides, forest clearings, on riverbanks and cultivated ground, hedges, tropical deciduous forest, in humid lowland forest, dry xeric forest, and montane cloud forest (Schaefer and Renner, 2011), this does not happen in Italy, and this is coherent with the results of our research. Furthermore, the higher competitiveness of the South American genotype entails a rapid growth, the production of a high dense canopy of leaves, late flowering and fruiting (Grime 2001). This is coherent with the fact that the genotype B in Camonica Valley climatic conditions produced less fruits late in the season, reaching difficultly ripeness. Conversely, the ability of producing a higher number of fruits (early in the season and reaching full ripeness) of the “Ciuenlai” is consistent with its more ruderal CSR strategy (Grime 2001). The genotype could have developed an ecological adaptation to the peculiar climatic conditions of Camonica Valley, different from the native area. This research confirm that CSR strategy analysis could be a useful instrument for the intraspecific characterization of landraces basing on their ecological features (Giupponi et al. 2019).

Historical information and strategy of valorisation

Caigua specie has been known in Europe for centuries: Feuillée (1714) called this plant *Momordica fructu striato laevi* (pre-Linnaeus plurinominal nomenclature) and Linnaeus (Linnaeus 2013) denominate it *Momordica pomis striatis*, informing that the species has striated fruits and come from Peru, and mentioned the work of Feuillée (Klien et al. 1989). In Italy, this species is traditionally cultivated only in few Alpine Valleys of Lombardy region. In this area, the vegetable takes the name of “Ciuenlai” (Bissanti 2020; Rossi et al. 2019; Giupponi et al. 2020), maybe referring to the exoticism of the fruit or to the “tail” shape, or “Milione”, “Milioncino”. (literally: “million, little million”), referring to the great productivity of the plant.

In Camonica Valley (Brescia province) the presence of this landrace is testified from at least three generation, and for sure it has been present since 1960 (Domini 2018; Rossi et al. 2019). Intriguingly, in the Andean region there are three varieties of Caigua, called “Criolla”, “Serrana” and “Italiana”, the latter producing a smaller fruit (Arroyo 1957). The Brazilian botanist Manoel Pio Correa, in 1975, reveals that he found unexpectedly the fruit of this plant for sale in Italian markets (Correa 1975).

In Italy, actually, the plant is considered an unconventional vegetable and the custodian farmer Oriana Belotti, interviewed during this research, described the traditional Italian way of eating Caigua, that is commonly pickled and stuffed with anchovies and cappers and never consumed as raw. The fruit is eaten once fully ripened, while no other parts of the plant are used as food. No mention was made about a traditional medicinal use of this plant.

Caigua has been cultivated by Oriana Belotti and the husband Giacomo Bontempi since 30 years at least, from self-propagated seeds, received in origin by a fellow villager, who called it “Ciuenlai”. The farmers used to serve the vegetable as appetizer in their restaurant, in Esine (Camonica Valley). The vegetable is although cultivated by hobby farmers in their gardens in other villages of the Valley, as Lozio, Ossimo, Cogno and Losine.

As many other landraces then, this unconventional vegetable is locally adapted and associated with traditional farming systems (Camacho Villa et al. 2005). Farmers plays a key role in the conservation of agrobiodiversity in biological, social and cultural interactions (Rudebjer et al. 2011). Although, cultural information and historical memory as well as multiplication, conservation and exchange of seeds between custodian farmers has always have been informal. In this sense, strategies to register and monitor landraces are important. Information on genetic resources is fundamental to set up priorities for their protection, breeding and enhancement. Collecting historical information (both bibliographic and direct testimonies) on the history and cultivation of “Ciuenlai” in Camonica Valley is necessary in fact to register this landrace in the European Register of Conservation Varieties (Spataro and Negri 2013), which is one of the main European tools for safeguarding plant agrobiodiversity.

Agrobiodiversity and especially herbaceous landraces are undergoing losses worldwide (Giupponi et al. 2020) and biodiversity conservation determined the set up of policies for agriculture and forestry in Europe’s biodiversity protection strategy. The adoption of a European Register of Conservation Varieties is one of the most important instruments for *in situ* conservation of landraces (Spataro and Negri 2013). The decline of genetic diversity, as well as the need of promoting and facilitating the use of traditional crop varieties, was confirmed also in the 2030 EU agenda, which states the necessity of reversing the trend of genetic erosion in agriculture by the conservation and use of traditional breeds and genotypes (European Commission 2020). With the recent law of 1st December 2015 n. 194 (“Provisions for the conservation and enhancement of biodiversity of agricultural and food interest”), Italy recognized the principles for the establishment of a national system of conservation and enhancement of biodiversity of agricultural and food interest, aimed at protecting local genetic resources from the risk of extinction and/or genetic erosion. This protection system provides for the creation of an Agrobiodiversity National Register, which was set up in December 2019 (Ministerial Decree 2019/39407; https://rica.crea.gov.it/APP/anb/) and has collected all information on landraces submitted by the regions to the Italian Ministry for Agriculture and Forestry (MiPAAF) in recent years. The first step, then, to enhance the cultivation of “Ciuenlai” would be the inscription to this Register.

A renewed interest for ancient landraces rich in bioactive compounds is growing. Landraces are unique resources for marginal and mountain territories (Giupponi et al. 2020), today subjected to abandonment (Cislaghi et al. 2019) and climatic change (Regione Lombardia 2016). They represent a possible starting point for unique high quality agri-food chains, that could be strategic for a sustainable and inclusive growth of mountain areas. Many underutilized species have potential for commercialization and the development of value chains that could increase the farmers income (Rudebjer et al. 2011), and often they have features interesting for improving nutrition and health. The “Ciuenlai” fruits showed interesting phytochemical and nutritional features, proving to be eligible as healthy products and functional food.

Besides fruits, also sprouts and leaves showed remarkable characteristics, as the content in saponins and the flavonoid fraction. Together with the fruit, Caigua leaves are included in the list of Annex 1 of the Ministerial Decree of 10 August 2018, indicating the plants allowed for herbal use and the preparation of extracts or other health products. Therefore, given the high content of secondary metabolites, the leaves could be interesting as a source of active ingredients or as herbal raw material, also due to the large aerial biomass that the plant produces and that is usually considered a waste product.

Channelling “Ciuenlai” in herbal industry can be then a strategy for its enhancement, besides valorising this product as typical food. For this last purpose, consortia and association of farmers and the cooperation with other stakeholders of the territory (such as restaurateurs) should be encouraged to enhance the use of landraces and preserve territorial traditions and history of agricultural and food practices.

Conclusions

This research allowed the acquisition of nutritional and phytochemical, agronomical and ecological as well as historical information on “Ciuenlai”. The “Ciuenlai” showed an interesting phytochemical and nutritional profile, proving to be a promising resource as raw material for the preparation of healthy products and functional food. The landrace of Camonica Valley showed a different phytochemical profile compared with the South American commercial Caigua with a significantly higher content of caffeoylquinic acid and lower content of saponins and flavanone and dihydroflavonols. Interestingly, the leaves and the sprouts of “Ciuenlai” resulted richer than the fruits in the two latter classes of compounds, meaning saponins and flavanone and dihydroflavonols. This result suggests that the aerial part of the plant could be eligible for herbal uses as well once performed further investigation. The herbal use of “Ciuenlai” could be a useful strategy for its valorisation, other than the promotion of this product as typical food. Another important aspect is that the plant, differently from the native genotype, is more ruderal and produces smaller and more numerous fruits, suggesting that it is an eco-morphotype different from the South American one, more adapted to the cultivation in Camonica Valley and other similar alpine valleys. This is favourable for its *in situ* (on farm) conservation and to start innovative value chains and opportunities of supplementary income for mountain farms.

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research complies to the ethical rules applicable for this journal.

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Figures

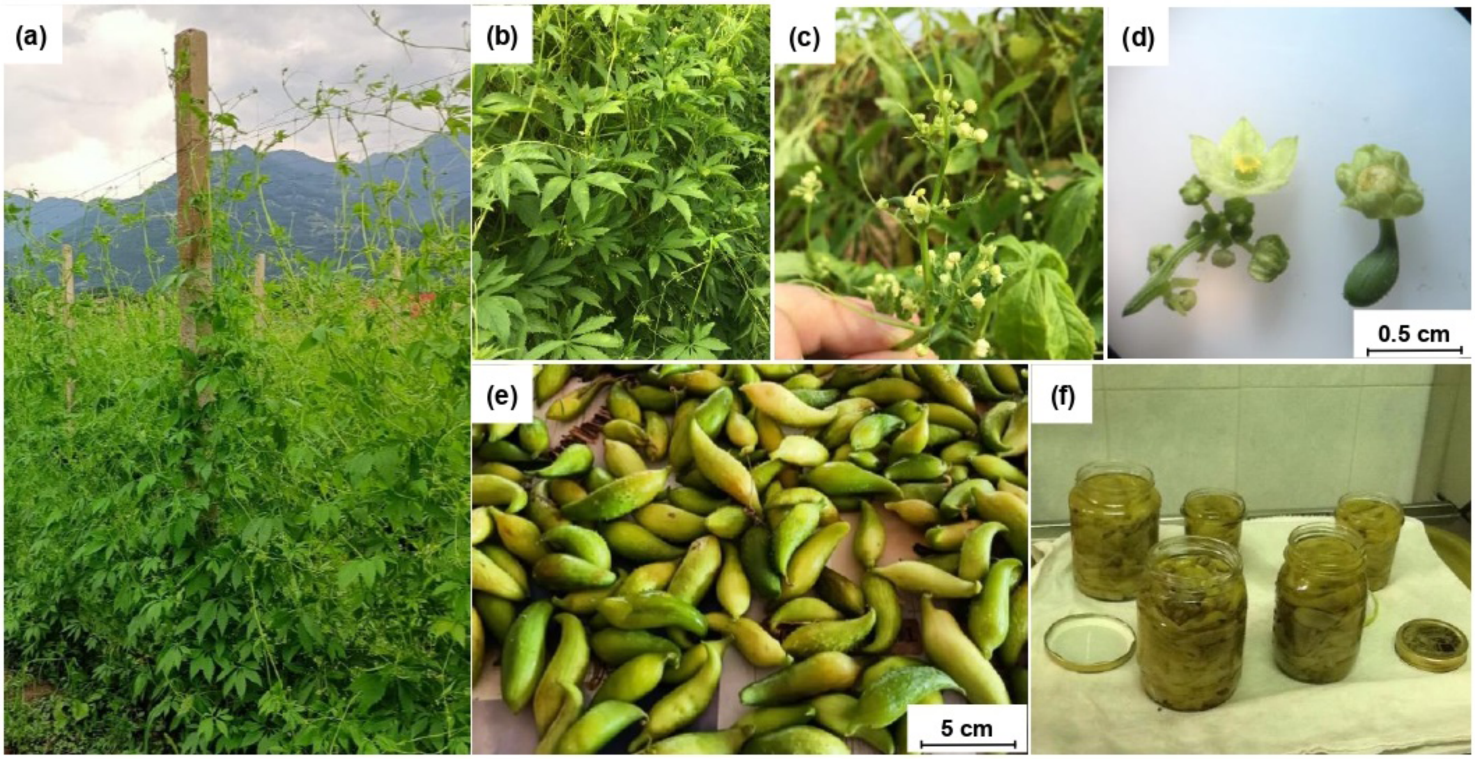

Figure 1

“Ciuenlai” landrace in the experimental field (**a**), detail of leaves and tendrils (**b**), detail of the inflorescence (**c**), detail of male (left) and female (right) flowers (**d**), ripen fruits (**e**), fruits pickled (**f**)

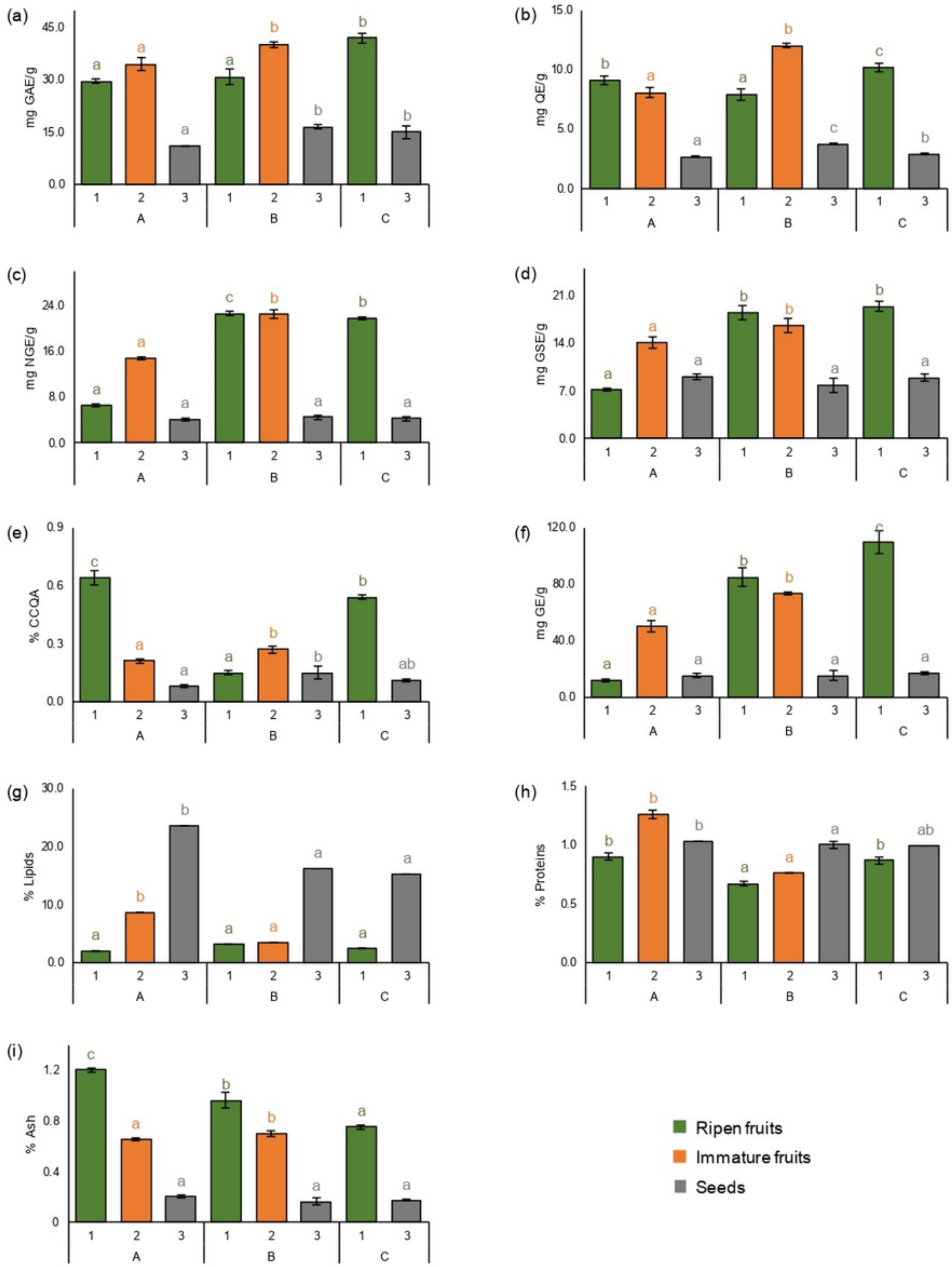

Figure 2

Phytochemical and nutritional analysis of fruits and seeds: **a**, total phenols content; **b**, total flavones and flavonols content; **c**, total flavanones and dihydroflavonols content; **d**, total saponins content; **e**, total caffeoylquinic acid content; **f**, total sugars content; **g**, total lipids content; **h**, total nitrogen content; **i**, total ash content. Different letters for the same colour column indicate statically significant difference ($p < 0.05$)

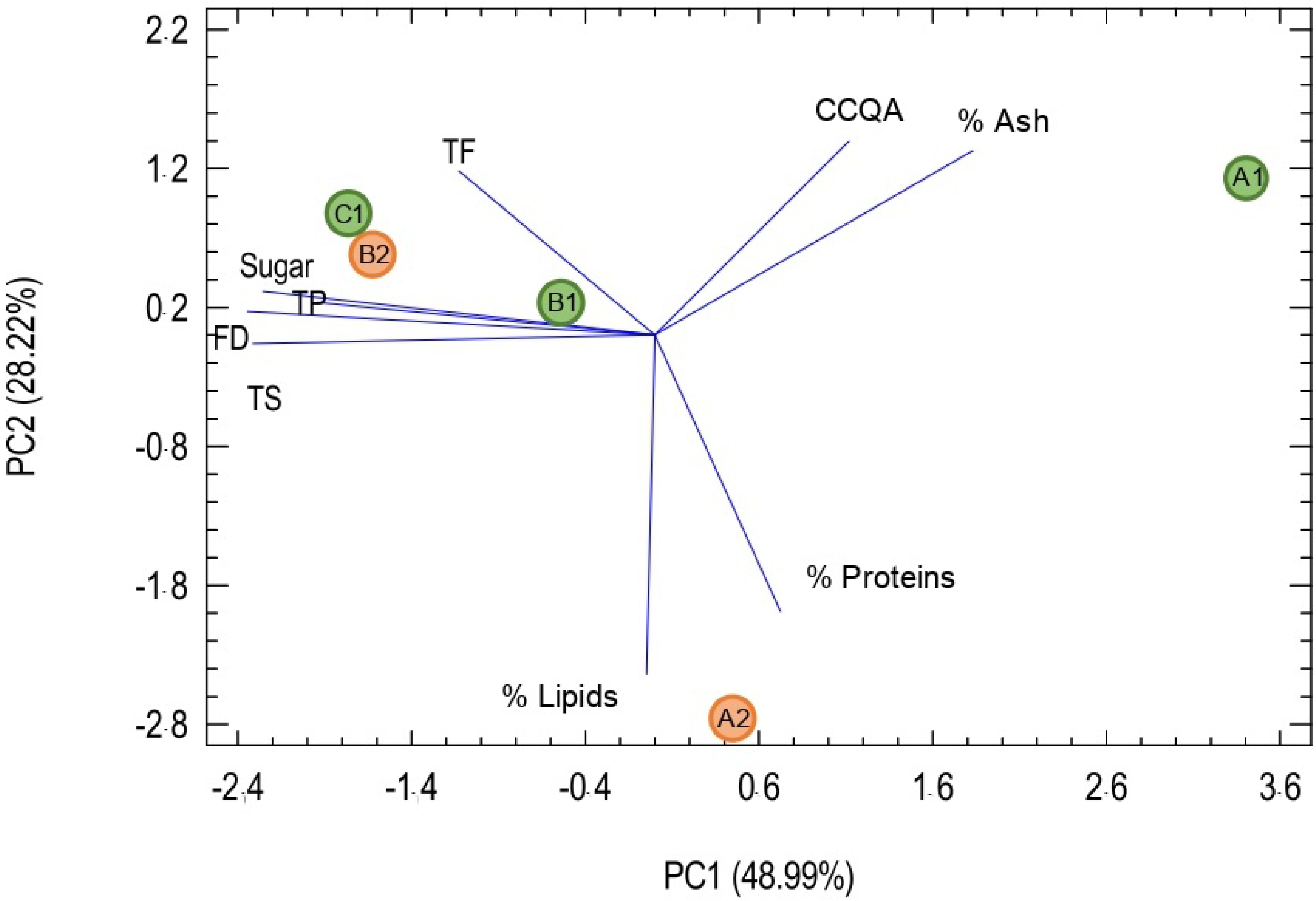

Figure 3

PCA biplot of samples (capital letters) associated with variables (lines). Key: A1, ripen fruit of “Ciuenlai”; A2, immature fruit of “Ciuenlai”; B1, ripen fruit of South American genotype; B2, immature fruit of South American genotype; C1, fruit of imported Peruvian Caigua; TF, total flavones and flavonols content; CCQA, total caffeoylquinic acid content; % Ash, total ash content; Sugar, total sugars content; TP, total phenols content; FD, total flavanones and dihydroflavonols content; TS, total saponins content; % Proteins, total nitrogen content, % Lipids, total lipids content

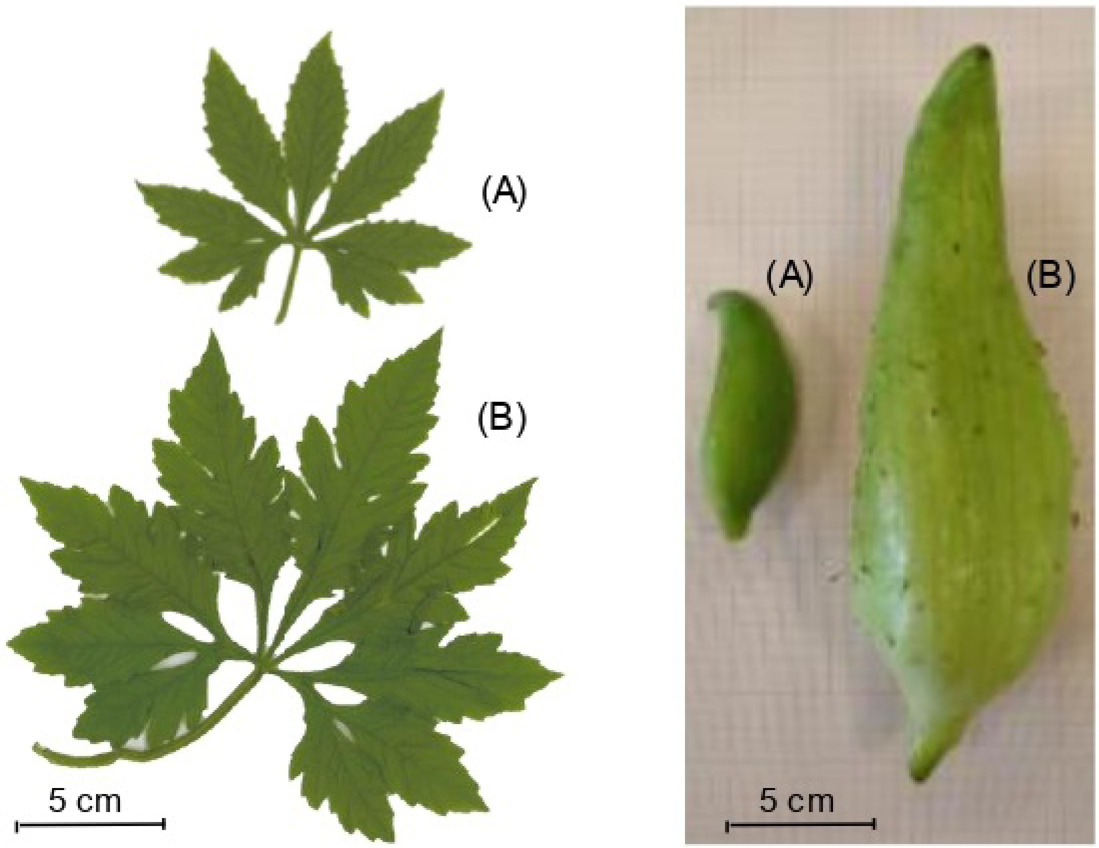

Figure 4

Comparison between leaves and fruits of “Ciuenlai” (**A**) and of South American genotype (**B**)

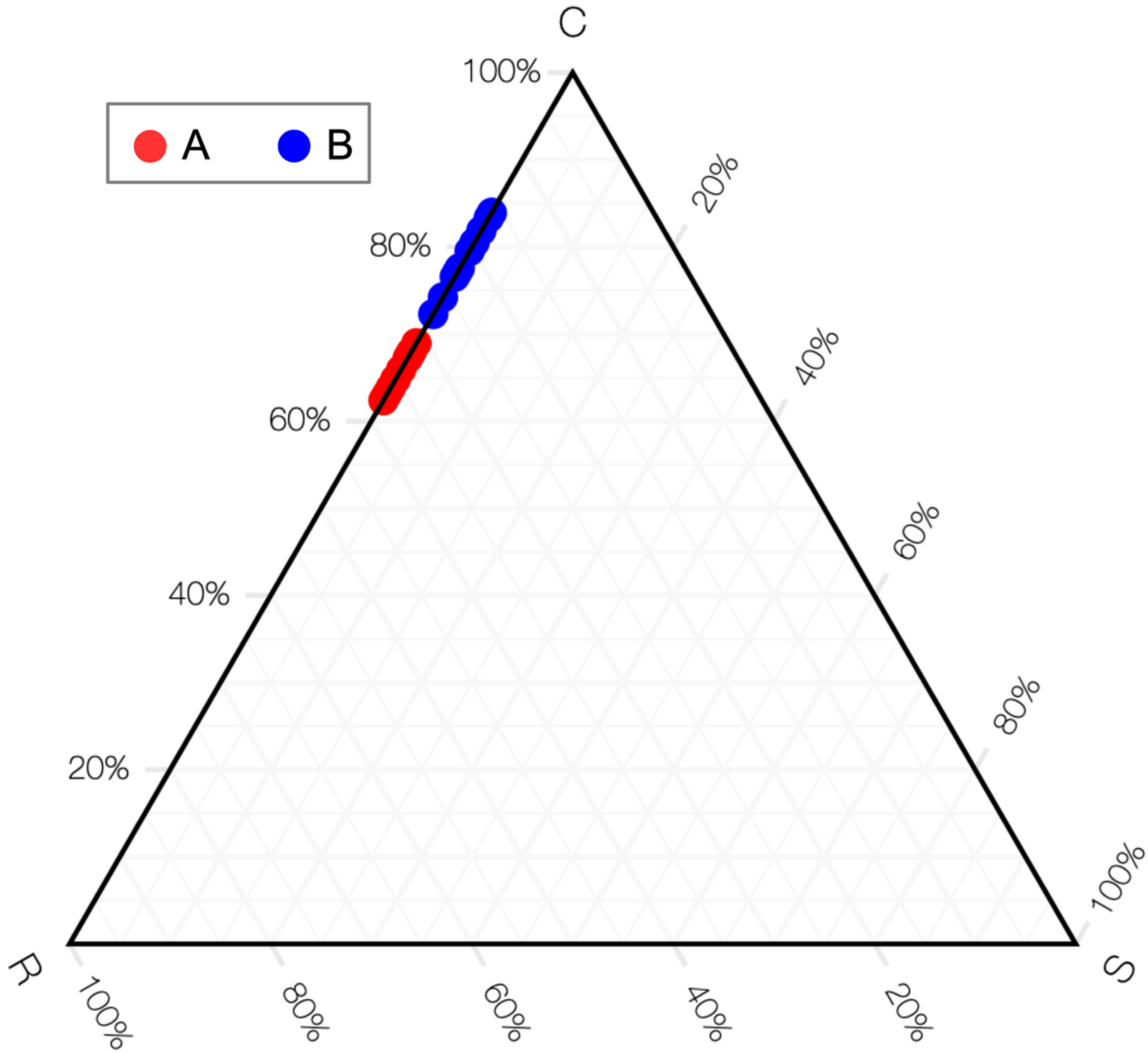

Figure 5

Comparison between leaves and fruits of “Ciuenlai” (**A**) and of South American genotype (**B**)